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Structural changes of Human Serum Albumin induced by binding to Ibuprofen

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Version 01

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Introduction

In this work, we present a label-free Flow-Induced Dispersion Analysis (FIDA)-based procedure for measuring absolute size and structural changes of Human Serum Albumin (HSA, 66.5 kDa) as it interacts with a small molecule drug molecule (Ibuprofen, 0.2 kDa). Intrinsic fluorescence detection of the tryptophan residue in HSA enables true native testing conditions, without any interference from immobilization or labelling with fluorescent dyes.

FIDA is a new capillary-based technology for measuring in-solution binding under native conditions and quantifying analyte concentration. FIDA is based on Taylor dispersion in a pressure driven flow of a ligand (termed indicator, e.g. HSA) interacting with the analyte of interest (e.g. Ibuprofen). The indicator alone appears small (i.e. it has a small hydrodynamic radius) when it is not bound to the analyte, but upon binding of the small drug molecule, the indicator will change conformation and thus appear larger (i.e. the complex has a larger hydrodynamic radius). The change in apparent size forms the basis for an accurate measure of analyte interaction and concentration.

Materials & Methods

FIDAlyzer instrument with 266 nm Laser-Induced Fluorescence detection (FIDA-Tech ApS). FIDA capillary (i.d.: 75 µm, LT: 100 cm, Leff: 88 cm). 67 mM phosphate buffer pH 7.4 was used as working buffer, HSA as indicator (0.5 mg/mL), and Ibuprofen (0-500 µM) as analyte.

Sample analysis was performed by filling the capillary with analyte, followed by injection of 39 nL indicator, which was mobilized towards the detector with analyte at 400 mbar.

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Results

The apparent size (hydrodynamic radius) of HSA was plotted as a function of Ibuprofen concentration (Figure 1). It was observed that an increase in Ibuprofen concentration led to an increase in the apparent size of HSA from 3.8 nm to 6.5 nm. t.

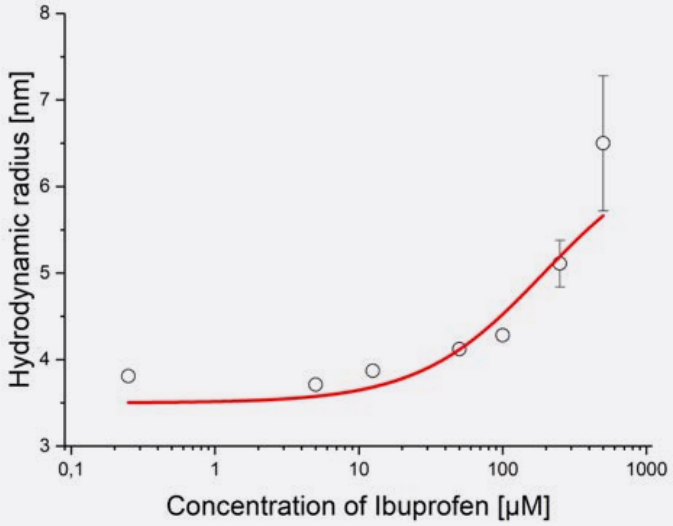

Figure 2: Apparent size (hydrodynamic radius) of HSA as function of Ibuprofen concentration. Binding isotherm fit (red line) resulted in a Kd of 104 µM.

The increase in apparent size of HSA indicated that Ibuprofen induced a structural change of HSA upon binding. A FIDA binding isotherm was fitted to the obtained data (red line in Figure 1), and the dissociation constant (Kd) for the HSA-Ibuprofen complex was estimated to 104 µM.

Conclusion

The FIDA methodology was applicable for measuring the binding of a small drug molecule to a significantly larger macro-molecule, under true native conditions, and revealed that a conformationel change was taking place. Furthermore, the use of intrinsic fluorescence detection enables simultaneous monitoring of fluorescence intensity induced by structural changes.

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